EXHIBIT 35

MOLECULAR CELL BIOLOGY

THIRD EDITION

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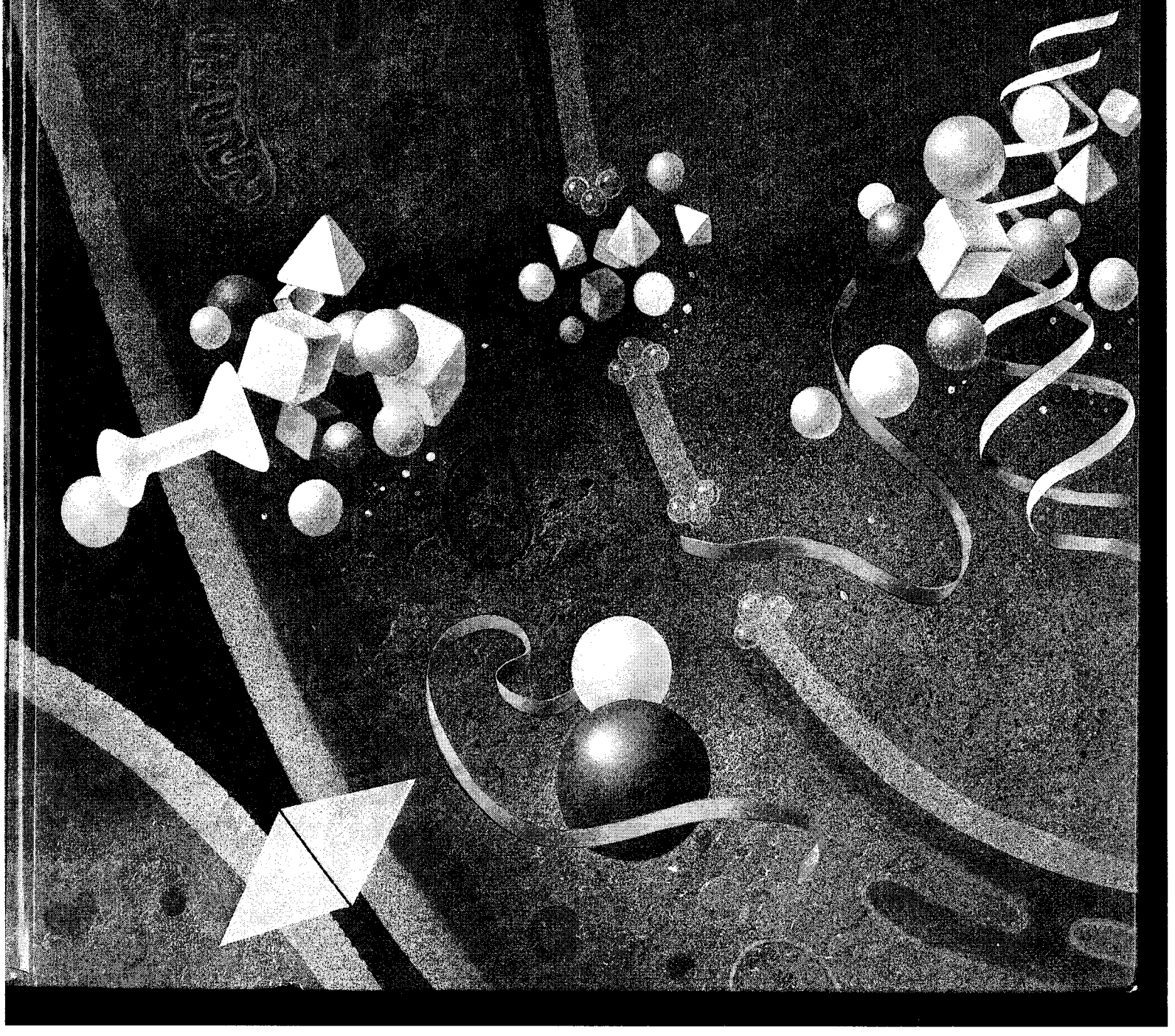

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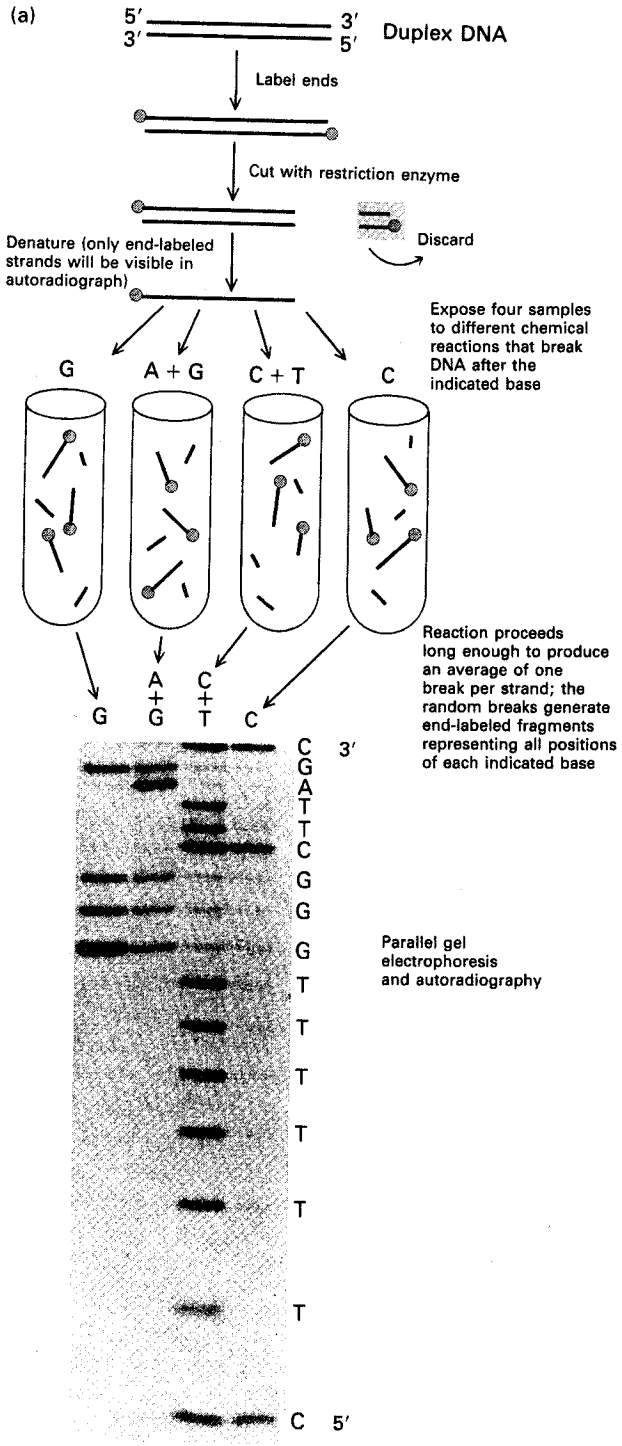
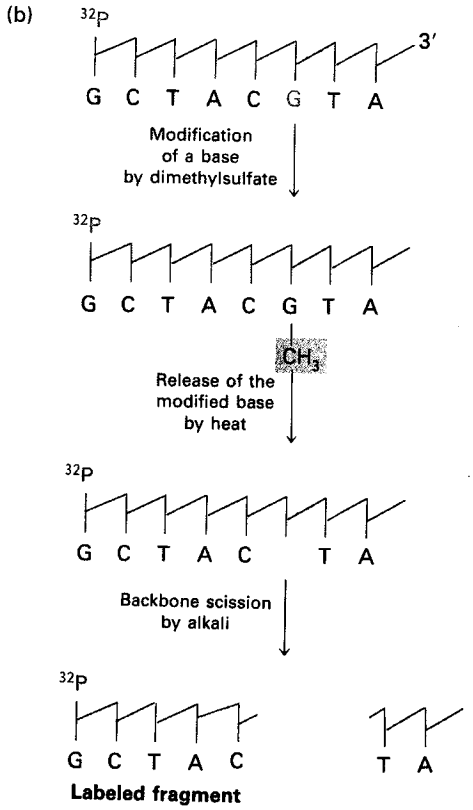
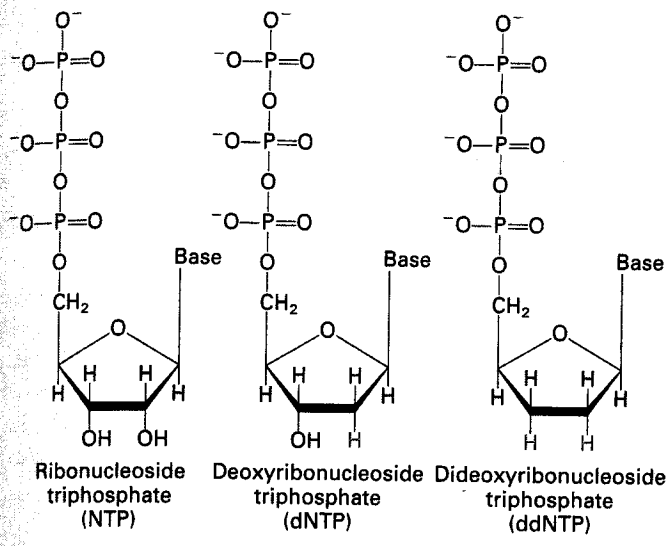




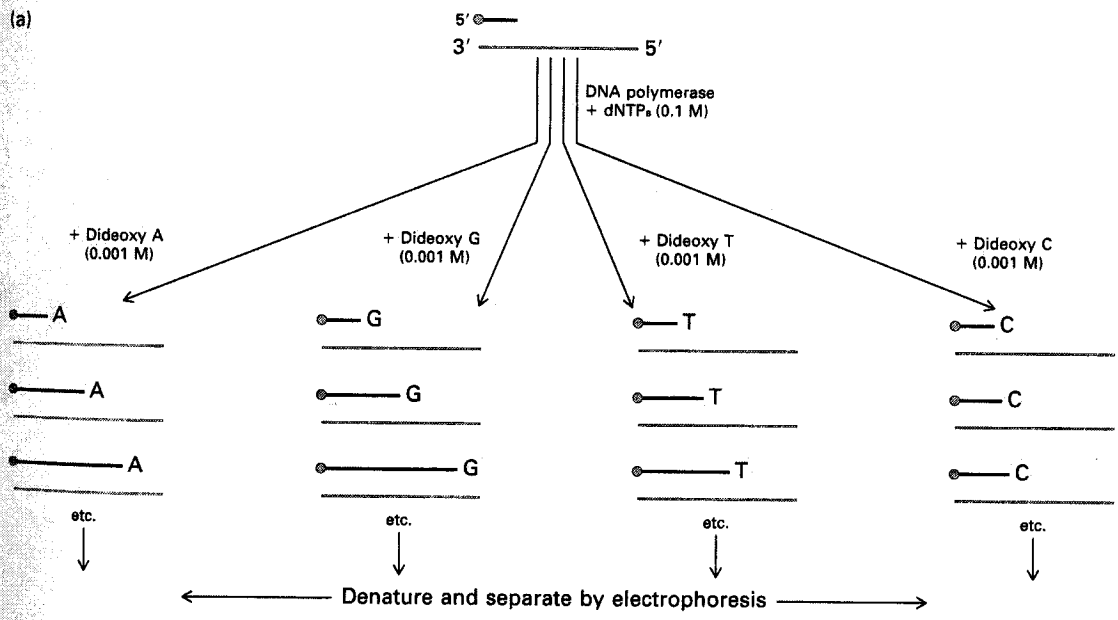
A FIGURE 7-28 Maxam-Gilbert method for sequencing DNA fragments up to ≈500 nucleotides in length. (a) The double-stranded fragment to be sequenced is labeled at the 5′ ends with ^{32}P (see Figure 7-20). The label (red circle) is removed from one end, and the fragment then is denatured. Four identical samples of the prepared fragment are subjected to four different chemical reactions that selectively cut the DNA backbone at G, G + A, C + T, or C residues. The reactions are controlled so that each labeled chain is likely to be broken only once. An example of the reaction that cleaves at a G is shown in (b). The labeled subfragments created by all four reactions have the label at one end and the chemical cleavage point at the other. Gel electrophoresis and autoradiography of each separate mixture yield one radioactive band for each nucleotide in the original fragment. Bands appearing in the G and C lanes can be read directly. Bands in the A + G lane that are not duplicated in the G lane are read as A. Bands in the T + C lane that are not duplicated in the C lane are read as T. The sequence is read from the bottom of the gel up. [See A. Maxam and W. Gilbert, 1977, *Proc. Nat'l. Acad. Sci. USA* **74**:560. Photograph from L. Stryer, 1988, *Biochemistry,* 3d ed., W. H. Freeman and Company, p. 120; courtesy of Dr. David Dressler.]

ddNTP terminates polymerization because the absence of a 3′ hydroxyl prevents addition of the next nucleotide. The mixture of terminated fragments from each of the four reactions is subjected to gel electrophoresis in parallel; the separated fragments then are detected by autoradiography. The sequence of the original DNA template strand can be read directly from the resulting autoradiogram (see Figure 7-30c). Once the sequence for a particular cloned DNA fragment is determined, primers for overlapping fragments can be chemically synthesized based on that sequence. The sequence of a long continuous stretch of DNA thus can be determined by individually sequencing the overlapping cloned DNA fragments that compose it.

Informatics: The Storage, Distribution and Analysis of DNA Sequence Data Vast amounts of DNA sequence have already been determined, and the pace at which new sequences are characterized is continuously accelerating. Computers are necessary to store and distribute this enormous volume of data. *Informatics* is the rapidly



◀ FIGURE 7-29 Structures of ribonucleoside triphosphate (NTP), deoxyribonucleoside triphosphate (dNTP), and dideoxyribonucleoside triphosphate (ddNTP).



▲ FIGURE 7-30 Sanger (dideoxy) method for sequencing DNA fragments. (a) A single strand of the DNA to be sequenced (blue line) is hybridized to a 5′-end–labeled synthetic deoxynucleotide primer. The primer is elongated in four separate reaction mixtures containing the four normal deoxynucleoside triphosphates (dNTPs) plus one of the four dideoxynucleoside triphosphates (ddNTPs) in a ratio of 100 to 1. A ddNTP molecule can add at the position of the corresponding normal dNTP, but when this occurs, chain elongation stops because the ddNTP lacks a 3′ hydroxyl. In time, each reaction mixture will contain a mixture of prematurely terminated chains ending at every occurrence of the ddNTP (yellow). (b) Three of the labeled chains that would be generated from the specific DNA sequence shown in the presence of ddGTP. (c) An actual audioradiogram of a polyacrylamide gel in which more than 300 bases can be read. Each reaction was carried out in duplicate using Sequenase™, a commercial preparation of the DNA polymerase from bacteriophage T7. [Part (c) courtesy of United States Biochemical Corporation.]

(b)

5′ ^{32}P-TAGCTGACTC 3′
3′ ATCGACTGAGTCAAGAGCTATTGGGCTTAA . . .

DNA polymerase
+dATP, dGTP, dCTP, dTTP
+ddGTP in low concentration

5′ ^{32}P-TAGCTGACTCAG 3′
3′ ATCGACTGAGTCAAGAGCTATTGGGCTTAA . . .
+
5′ ^{32}P-TAGCTGACTCAGTTCTCG 3′
3′ ATCGACTGAGTCAAGAGCTATTGGGCTTAA . . .
+
5′ ^{32}P-TAGCTGACTCAGTTCTCGATAACCCG 3′
3′ ATCGACTGAGTCAAGAGCTATTGGGCTTAA . . .

(c)